

acids 382 to 429 of SEQ ID NO:2; about amino acids 438 to 490 of SEQ ID NO:2; about amino acids 495 to 547 of SEQ ID NO:2. A type 1 repeat of TSP-2 may have one or more of the following activities: (i) may bind the membrane protein CD36; (ii) may promote an inhibitory effect of TSP-2 on endothelial cell migration; (iii) may induce cell apoptosis, e.g., endothelial cell apoptosis; (iv) may have anti-angiogenic activity of TSP-2; or (v) may inhibit unwanted cell proliferation, e.g., a benign or malignant unwanted cell proliferation, e.g., tumour growth. In a preferred embodiment, a TSP-2 peptide is about 4, 5, 6, 7, 8, 10, 15, 20 or 50 amino acids in length and contains a sequence which inhibits endothelial cell migration. For example, the peptide can include a PWAEW sequence (about amino acid residues 386 to 390 of SEQ ID NO:2), or the fragment can include a WSPWAEW sequence (about amino acids 384 to 390 of SEQ ID NO:2), or conservative substitutions of either sequence. Other peptides can include 4, 5 or 6 amino acids from a WSPWAEW (SEQ ID NO:10) sequence or conservative substitutions thereof. In another embodiment, a TSP-2 peptide includes about 5 to 50 amino acids of the type 1 repeat of TSP-2, or about 5 to 50 amino acids of TSP-2 sequence on one or both sides of the type 1 repeat. In a preferred embodiment, the fragment is 4, 5, 6, 7, 10, 15, 20 or 50 amino acids in length and contains a sequence which contains a receptor binding sequence, e.g., a CSVTVG (SEQ ID NO:11) sequence, which binds CD36.

Replace the paragraph beginning at page 21, line 9, with the following rewritten paragraph:

-In another aspect, the invention features a TSP-2 antibody. The antibody can be a polyclonal or a monoclonal antibody. The antibody can be raised, e.g., against the intact protein or a fragment thereof. In one embodiment, the antibody can bind specifically to a TSP-2 protein or a fragment. In another embodiment, the antibody binds TSP-2 with significantly greater affinity than TSP-1, e.g., 10%, 20% or 50% higher affinity. In a preferred embodiment, the TSP-2 epitope can be a 10, 15, 20 or 30 amino acid peptide of SEQ ID NO:2, e.g., the epitope is a 15-amino acid peptide DKDTTFDLFSISNIN (SEQ ID NO:3). In another preferred embodiment, the epitope can overlap the 15-amino acid peptide epitope of DKDTTFDLFSISNIN (SEQ ID NO:3).

Replace the paragraph beginning at page 28, line 17, with the following rewritten paragraph:

Using these methods, full-length TSP-2 has been obtained but the protein yields have been relatively low. Therefore, 293 human embryonic kidney cells were transfected with a different human TSP-2 expression vector. A PCEP4 vector (Invitrogen) was used that was modified as follows: a BM 40 signal peptide sequence was introduced in front of the insertion site of TSP-2, the antibiotic selection gene was replaced with a puromycin gene for fast and efficient antibiotic selection of stably transfected clones, and a total of 8 histidin residues at the C-terminal end have been included to facilitate purification of the recombinant protein. Using this vector, stably transfected 293 cells produce high amounts of the recombinant protein and the use of mammalian cells ensures efficient glycosylation of recombinant TSP-2. Four different recombinant TSP-2 proteins have now been expressed. Construct I expresses selectively the N-terminal procollagen domain of TSP-2 (nucleotides 294-1367), the region with the least homology to TSP-1. Construct 2 expresses, in addition, the type I repeats (nucleotides 294-1883) which contain several biologically active sites including two CSVTCG (SEQ ID NO:11) sequences that mediate binding to the CD36 receptor on endothelial cells. Construct 3 expresses the type I repeats (nucleotides 1383-1883) only. Construct 4 expresses the full-length mature TSP-2 molecule, excluding the signal peptide (nucleotides 294-3755) which is provided in the expression vector. Such recombinant proteins can be used for the generation of monoclonal anti-TSP-2 antibodies, for the establishment of a human TSP-2 ELISA, and for the systemic treatment of experimental tumors.

Replace the paragraph beginning at page 40, line 2, with the following rewritten paragraph:

The following synthetic peptides, derived from the amino acid sequence of human TSP-2, were synthesized:

Peptide 1: RESHFRGLLQNVHLVF: procollagen domain, AA 207-222 (SEQ ID NO:6)

Peptide 2: PATCANPSFVEGECCPSC: procollagen domain, AA 366-383 (SEQ ID NO:7)

Applicant : Michael J. Detmar et al.  
Serial No. : 09/536,087  
Filed : March 24, 2000  
Page : 4

Attorney's Docket No.: 10287-051001 / MGH 1470.0

Peptide 3: FAENETWVVDSCTTCTCKKFKT: procollagen domain, AA 336-357 (SEQ ID NO:8)

*A 4* Peptide 4: ELIGGPPKTRNMSAC: procollagen domain, AA 315-329 (SEQ ID NO:9)

Peptide 7: WSPWAEW: first type I repeat, AA384-390 (SEQ ID NO:10) *f*

Replace the paragraph beginning at page 40, line 9, with the following rewritten paragraph:

*A* *5* HDMEC migration experiments were performed essentially as described above.  $1 \times 10^5$  HDMEC were added to the upper chamber in 300  $\mu$ l of DMEM medium, or in DMEM medium containing 10 Mol/l of the synthetic peptides. All media were supplemented with 10 mg/ml BSA. As shown in Figure 7, in DMEM medium,  $212 \pm 12$  HDMEC/mm<sup>2</sup> migrated to the underside of the inserts (C; column 1). Peptides 1, 2, 3, and 4 did not significantly modify HDMEC migration. Peptide *7* (WSPWAEW; SEQ ID NO:10) inhibited HDMEC migration by 47.6% ( $111 \pm 39$  HDMEC/mm<sup>2</sup>, column 2). These results reveal an important role of this TSP-2 specific peptide for the anti-angiogenic activity of TSP-2. Importantly, this peptide is distinct from the CSVTCG (SEQ ID NO:11) sequence that has been described to bind to the CD36 receptor on endothelial cells. Dawson et al. (1997) *J. Cell. Biol.* 138:707-717. All assays were performed in quadruplicate. *f*